Influence of visual and olfactory cues on field trapping of the pollen beetle, *Astylus atromaculatus* (Col.: Melyridae)

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Abstract

Field trapping experiments investigated the response of the pollen beetle *Astylus atromaculatus* to visual and olfactory cues during a 3-year period, 1999–2001. The visual preference of the pollen beetle was determined using yellow, white, blue, green and red water traps. The yellow trap was most attractive, capturing 56% of the total beetles trapped, with 30% caught by the blue and white traps, while 14% was caught by the red and green traps. The response of the beetle to olfactory cues was then evaluated by using the yellow water trap with three antennally active components identified in the volatiles of sorghum panicles by coupled gas chromatography (GC)–electroantennographic detection and GC–mass spectrometry. These components were 2-phenylethanol, benzyl alcohol and linalool. There were no significant colour × chemical compound interactions and traps baited with 2-phenylethanol captured significantly more beetles than unbaited traps, irrespective of trap colour, demonstrating the effectiveness of olfactory cues in trapping the pollen beetle. Traps baited with 2-phenylethanol were more attractive than and caught more beetles than traps baited with linalool. 2-Phenylethanol had the greatest effect on the relatively unattractive blue trap, confirming the importance of olfactory cues mediating *A. atromaculatus* attraction.

Introduction

The pollen beetle, *Astylus atromaculatus* Blanchard (Col.: Melyridae), is indigenous to South America, where adults have been observed feeding on the pollen of rice, sorghum and cotton in Brazil, Argentina and Bolivia (Chiesa-Molinari 1964; Venica 1969; Huddleston et al. 1972). Although *A. atromaculatus* was first found in South Africa in 1916, for unknown reasons it has only recently been considered as a pest, with both adults and larvae causing economic losses on maize (*Zea mays* L.) and grain sorghum (*Sorghum bicolor* L. Moench). It is not known why this pest has not spread to other southern African countries. In South Africa, *A. atromaculatus* is univoltine, with the adult beetles emerging from the soil in mid-December. Their numbers increase rapidly, peaking in January and February. Newly emerged beetles aggregate and hang in clusters on leaves of young maize plants, certain grasses and weeds, and invade maize and sorghum fields as plants commence tasseling and pollen becomes available (Drinkwater 1998). Adults are also voracious feeders on sorghum pollen and flowering parts, resulting in damage to the spikelets, poor pollination and physical damage to kernels, all of which result in chaffy panicles and reduced yield. The larvae feed on dry seed in the soil causing more damage than the adults, particularly to maize, sorghum, sunflower, groundnut and cotton, resulting in serious stand loss and in some cases necessitating replanting of complete fields (Drinkwater 1998). No monitoring strategy has been developed for this pest and the relationship between beetle numbers and larvae

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infestation levels and damage to crops have not been determined yet. In addition to damaging crops, ingestion of large numbers of beetles causes diarrhoea and death in cattle grazing in infested fields (Drinkwater 1998).

Astylus atromaculatus is currently controlled by the use of broad spectrum insecticides (Krause et al. 1999), which has serious drawbacks including the potential for the beetle to develop resistance against these insecticides and environmental and grain contamination. Consequently, an alternative strategy is needed that can be used in combination with the use of seed treatments with insecticides against larvae, possible trapping of beetles or accurate timing of insecticide sprays against beetles.

Visual and olfactory cues have been identified for another pollen beetle, *Meligethes aeneus* (F.) (Col.: Nitidulidae), a pest of oilseed rape in the UK. In field studies, *M. aeneus* was attracted to yellow traps compared with yellow-green and white traps and this attraction was significantly enhanced by the addition of isothiocyanates and some other host plant volatiles (Blight and Smart 1999). The attraction of *A. atromaculatus* to coloured discs in a glass arena has been studied by Esterhuizen (1997), but the beetle's responses to traps baited with host plant-derived volatiles has not been reported. In this paper, field-trapping experiments investigate the effects on capture of *A. atromaculatus* of different coloured traps with or without host-derived volatiles, with the aim of: (i) understanding the interaction between visual and olfactory cues on responses of the beetle and (ii) developing an efficient trap lure that can be used in its integrated management.

Materials and Methods

Source of plants

Plants of *S. bicolor* (Poaceae) (cv. Serredo) were grown individually in soil-filled pots and were watered every other day in a glasshouse at 25°C with a 16 h day length, at Rothamsted, UK. Panicles were allowed to emerge and then volatiles were collected from them while still on the intact plant.

Collection of volatiles

Entrainment of volatiles was performed on intact growing sorghum panicles using portable equipment developed at Rothamsted (Agelopoulos et al. 1999). The sorghum panicle (~30–50% flowering) was enclosed in a customized open-bottomed glass vessel (100 mm diameter × 300 mm length) and the bottom was closed with two semicircular aluminium plates that fitted loosely around the stem. Any large gaps between the plates and the stem were packed with silanized and baked glass wool. Air, purified by passage through an activated charcoal filter, was pumped up through the vessel via a port in one of the aluminium plates, and volatiles were then collected on Porapak absorbent tubes in collection ports at the top of the vessel. By controlling the flow rates so that more clean air was pumped in than was drawn out, the risk that unfiltered air would be drawn into the vessel from outside was avoided, whilst obviating the need for an injurious tight seal around the stem. Typical flow rates used were 1000 ml/min in and 700 ml/min out. All connections were made using polytetrafluoroethylene tubing and ferrules and glassware, was heated at 180°C for at least 2 h before use. Volatiles were collected on thermally conditioned Tenax® (Supelco, Bellefonte, PA, USA) or Porapak Q traps (50 mg each), packed between two glass wool plugs contained in glass tubes. Controls were volatiles collected from the glass vessel only. All experiments were replicated at least three times.

Analysis of volatiles

Tenax® traps were analysed by thermal desorption on a HP 5890 Series II gas chromatograph fitted with an Optic programmed temperature vaporization (PTV) ATAS Optic 2 programmable injector (ATAS, Veldhoven, the Netherlands), a HP-WAX capillary column (30 m × 0.23 mm ID × 0.5 μm film thickness), and a flame ionisation detector (FID) detector. The PTV unit was temperature programmed for a rapid rise from an initial injection temperature of 30°C to 220°C in 30 s. The oven temperature was programmed as follows: 30°C for 5 min, 10°C/min to 220°C for 30 min. The carrier gas was nitrogen.

Porapak tubes were eluted with freshly distilled diethyl ether (500 μl) and 1 μl was analysed by gas chromatography–electroantennographic detection (GC–EAD). The GC–EAD system, in which the effluent from the GC column is simultaneously directed to the antennal preparation and the GC detector, has been described previously (Wadhams 1990). Separation of the volatiles was achieved on an AI 93 GC equipped with a cold on-column injector and an FID. Two columns, a 50 m × 0.32 mm i.d. HP-1 column and a 30 m × 0.3 mm i.d. HP-WAX column were used for the analysis to confirm the identities of candidate EAG-active compounds. For the HP-1

column, the oven temperature was maintained at 40°C for 2 min and then programmed at 5°/min to 100°C and then at 10°/min to 250°C. For the HP-WAX column, the oven temperature was maintained at 40°C for 1 min and then programmed at 10°/min to 220°C. The carrier gas was hydrogen. An antenna was excised and suspended between two electrodes. The tip of the terminal process of the antenna was removed to ensure a good contact. GC–EAD analyses were replicated using three separate individual antennae of the beetle. The outputs from the EAD amplifier and the FID were monitored simultaneously and analysed using a software package (UN-06; Syntech, Hilversum, the Netherlands).

Coupled GC–mass spectrometry was carried out on a VG Autospec, Fisons Instruments Ltd (Crawley, UK), fitted with a HP-1 capillary column (50 m × 0.32 mm i.d. HP-1) attached to an on-column injector. Ionization was by electron impact at 70 eV, 250°C. The oven temperature was maintained at 30°C for 5 min and then programmed at 5°/min to 250°C. Tentative identification by GC–MS was confirmed by peak enhancement with authentic samples.

Chemicals

Hexanal, heptanal, octanal, 6-methyl-5-hepten-2-one, nonanal, decanal, benzaldehyde, linalool, phenylacetaldehyde, acetophenone, 2-ethylbenzaldehyde, 4-ethylbenzaldehyde, methyl salicylate, 2-ethylacetophenone, 4-ethylacetophenone, 2-phenylethanol, benzyl alcohol and phenol were purchased from Lancaster Synthesis Ltd, Eastgate, UK. Purities ranged from 98% to 99.5%.

Chemicals that were tested in the field were dispensed through diffusion from polyethylene bags. Undiluted compounds were individually applied to small pieces of cellulose sponge, which were then heat sealed into polyethylene tubing (Smart and Blight 2000).

Field experiments

Three field experiments (A–C) were conducted at the ARC-Grain Crops Institute in Potchefstroom, South Africa, during February of the 1999, 2000 and 2001 seasons. Each experiment lasted 5 or 6 days, depending on the number of replicates in each experiment. Each experiment comprised a randomized block design. The traps consisted of plastic bowls (20 cm wide × 8 cm deep) of different colours (red, white, yellow and green). Traps were purchased from a local supermarket. Each bowl was filled to about two-thirds full with a 1% solution of Tween 80 to hold trapped beetles. One row of traps represented one replicate and contained one trap with each treatment. Traps were set out in a straight line with a 10 m inter-trap spacing in a flowering sorghum field. The sorghum field was 80 × 100 m and the first trap was situated 10 m from the side of the field. The traps were set up at the height of the panicles and were suspended from a metal frame that carried anti-hail netting above the sorghum plot. Trapped insects were removed daily and then the traps were re-randomized to the next replicate of the design.

Experiment A: Effect of visual cues on trap captures

The purpose of this experiment carried out in 1999 was to test the alighting preference of *A. atromaculatus* for different colours. Yellow, white, blue, green and red water traps were prepared as described above in the experimental design. Each trap was replicated five times. Trapped insects were removed daily and counted.

Experiment B: Comparison of yellow traps baited and unbaited with sorghum panicle volatiles

This experiment which was carried out during 1999 and 2000 evaluated the response of *A. atromaculatus* adults to yellow water traps baited with three antennally active sorghum panicle volatiles released at approximately the same rate in the field: benzyl alcohol (3 mg/day), 2-phenylethanol (2.6 mg/day) and linalool (3 mg/day). Trap catches were compared to an unbaited control trap. The experiment lasted 5 days with one replicate being carried out per day.

Experiment C: Comparison of different coloured traps baited and unbaited with 2-phenylethanol

This experiment was carried out during 2000 and 2001 investigated colour–olfactory cue interactions. On the basis of data we obtained from Experiment B, yellow, white and blue water traps baited with or without 2-phenylethanol were tested. Two traps of each colour (one baited and the other not) were used in each of the six replicates. The experimental design and trap placement was similar to what was described above. Trapped insects were removed daily and counted.

Data analyses

Beetle numbers were $\log_{10}(x + 1)$ transformed before analyses and data analysed using Statsgraphics Plus 5 (2000). In experiments A and B one-way analyses of variance (ANOVA) were performed. Data were

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analysed separately for the two seasons in Experiment B. Transformed treatment means were compared at P = 0.05 using Tukey's honestly significant difference (HSD) procedure.

Multi-factor ANOVAs were used to determine if there were interactions between colour and bait in Experiment C. Data were analysed separately for the two seasons and transformed treatment means were compared at P = 0.05 using Tukey's HSD procedure. The Behrens–Fisher *t*-test was then used to compare the means of each colour trap with and without the lure. Means were then transformed back and are provided in figures.

Results

Analysis of volatiles

Coupled thermal–desorption–GC–MS analysis of headspace volatiles from intact sorghum and panicles (30–50% flowering) revealed consistently alcohol, aldehyde and keto-derivatives of benzene identified as 2-ethylbenzaldehyde, 4-ethylbenzaldehyde, 2-ethylacetophenone, 4-ethylacetophenone, phenylacetaldehyde, 2-phenylethanol, 4-isopropylbenzylalcohol, 1,3-diacetylbenzene and 1,4-diacetylbenzene (fig. 1). With the exception of 2-ethylbenzaldehyde and 2-ethylacetophenone, comparison of retention times and GC–MS analysis of authentic samples on non-polar HP-1 and polar HP-WAX columns confirmed the identities of these compounds in the volatiles.

Coupled GC–EAD located a number of physiologically active components, including hexanal, benzaldehyde, octanal, benzyl alcohol, 2-phenylethanol, phenylacetaldehyde and linalool in the volatiles of sorghum panicles (fig. 2), which were tentatively identified by GC–MS and confirmed by co-injection with authentic compounds on both polar and non-polar phases.

Field experiments

In general, all the traps caught predominantly pollen beetles. However, a few species of Diptera, Coccinellidae beetles and aphids were also caught in the traps. Traps captures of these insects were relatively insignificant compared with the captures of pollen beetles.

Experiment A: Effect of visual cues on trap captures

Astylus atromaculatus preferred yellow traps to red and green ($F = 10.10$; d.f. = 4, 20; $P < 0.0001$) (fig. 3). A total of 1038 beetles were caught in this

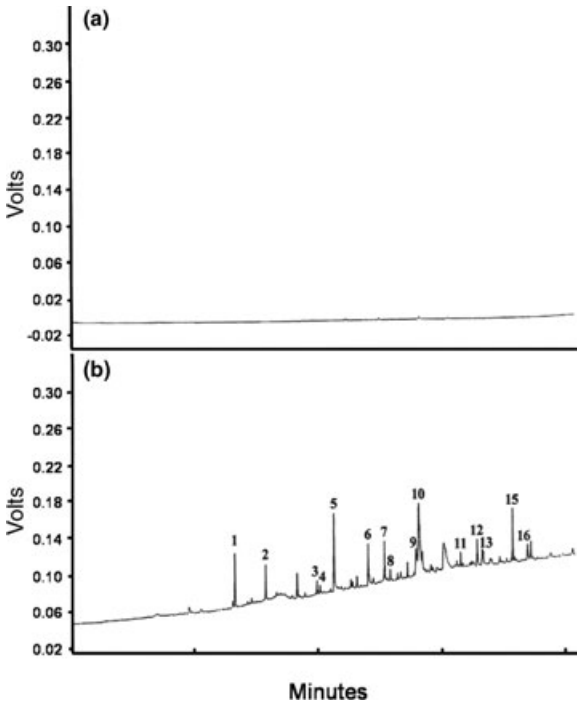

Fig. 1 Representative gas chromatography (GC) profiles of volatiles emitted by sorghum panicles (30–50% flowering) trapped on Tenax and analysed by thermal desorption on a HP-WAX column. (a) Blank, entrainment chamber only and (b) sorghum (Serredo variety), 1 = hexanal, 2 = heptanal, 3 = octanal, 4 = 6-methyl-5-hepten-2-one, 5 = nonanal, 6 = decanal, 7 = benzaldehyde, 8 = linalool, 8 = phenylacetaldehyde, 9 = acetophenone, 10 = 2-ethylbenzaldehyde, 11 = 4-ethylbenzaldehyde, 12 = methyl salicylate, 13 = 2-ethyl acetophenone, 14 = 4-ethylacetophenone, 15 = 2-phenylethanol + benzyl alcohol, 16 = phenol.

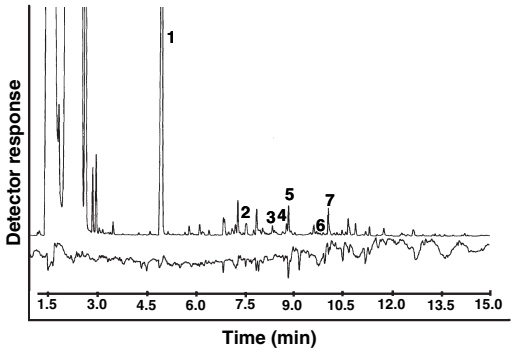

Fig. 2 Gas chromatography–electroantennographic detection (GC–EAD) chromatogram of volatiles of sorghum panicles (~30–50% flowering) on a HP-1 column. Some EAD-active components are indicated by the peaks including the three components tested in the field: 1, hexanal; 2, benzaldehyde; 3, octanal; 4, benzyl alcohol; 5 phenylacetaldehyde; 6, linalool; 7, 2-phenylethanol.

experiment, 56% of which were found in the yellow traps. However, because of the variability in trap catch between replicates, there was no significant difference between numbers caught in yellow, white and blue traps. These traps were significantly more attractive than red or green traps.

Experiment B: Comparison of yellow traps baited and unbaited with sorghum panicle volatiles

The number of beetles captured in traps baited with sorghum panicle volatiles were higher in 1999 (total 19 269) than in 2000 (total 8839) (fig. 4). 2-Phenylethanol baited traps caught significantly more beetles than the linalool and unbaited traps during 1999 ($F = 14.80$; d.f. = 3, 16; $P = 0.0001$) (fig. 4). Although a similar trend was observed in 2000 only traps baited with 2-phenylethanol captured significantly higher numbers of beetles than the unbaited traps ($F = 3.30$; d.f. = 3, 16; $P = 0.035$). The 2-phenylethanol baited traps provided highest catches, with 32% and 37% of the total trap catches for 1999 and 2000 respectively.

Experiment C: Comparison of different coloured traps baited and unbaited with 2-phenylethanol

There were no significant interactions between trap colour and compound in any of the two seasons (season 1: $F = 0.73$; d.f. = 2, 30; $P = 0.4903$; season 2: $F = 2.15$; d.f. = 2, 30; $P = 0.1345$). Trap colour did however have a significant effect on the number of beetles captured in season 1 ($F = 24.93$, d.f. = 2, 30; $P = 0.00001$) and season 2 ($F = 3.38$; d.f. = 2, 30; $P = 0.0473$). The addition of 2-phenylethanol baited lures resulted in significant increases in the numbers of captured beetles in baited traps in season 1 ($F = 17.58$, d.f. = 2, 30; $P = 0.0002$) and season 2 (58.58, d.f. = 2, 30; $P = 0.00001$).

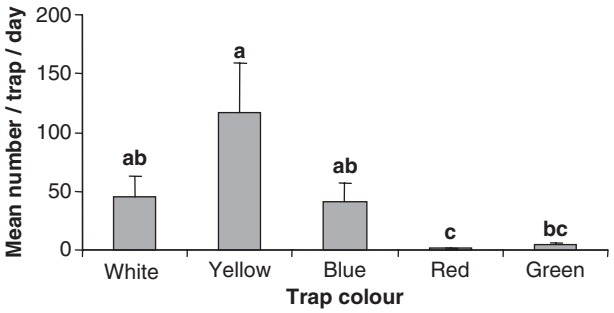

Fig. 3 Backtransformed mean number of *Astylus atromaculatus* in unbaited coloured water traps. Letters above bars indicate significant differences at $P < 0.05$ (Tukey's honestly significant difference). Bars represent standard errors.

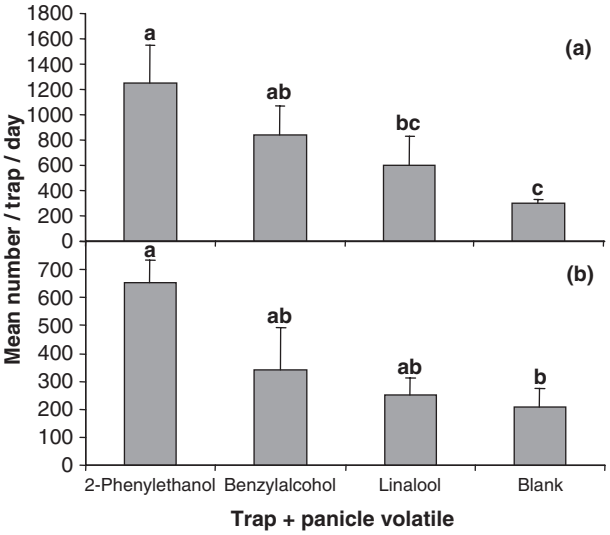

Fig. 4 Backtransformed mean number of *Astylus atromaculatus* in yellow water traps baited with volatiles from sorghum panicles (a = 1999 season; b = 2000 season). Letters above bars indicate significant differences at $P < 0.05$ (Tukey's honestly significant difference). Bars represent standard error.

Except for the yellow traps in the 2000 seasons, all baited traps caught significantly more beetles than their unbaited counterparts in both seasons (fig. 5). A total of 7836 beetles were caught and catches in baited traps increased proportionally by 48%, 59% and 73% for yellow, blue and white traps respectively compared with the equivalent unbaited traps. Many more beetles were captured during 2001(total 17 905) and the catches in the baited traps increased proportionally by 63%, 84% and 73% for yellow, blue and white respectively when compared with the equivalent unbaited traps.

Discussion

Coupled thermal–desorption–GC–MS analysis revealed the volatile profiles of the intact flowering panicles of sorghum even though the entrainment period was rather short (1 h). The effectiveness of thermal desorption has been demonstrated previously for the analysis of volatiles entrained from small samples for relatively short periods (Agelopoulos et al. 1999), and also in the present study using sorghum flowering panicles. The GC–MS analysis showed that volatiles of flowering panicles were benzene derivatives in agreement with results obtained on the analysis of floral volatile emissions from several plant species (Knudsen et al. 1993).

The field trapping experiments showed that there was no interaction between trap colour and chemical

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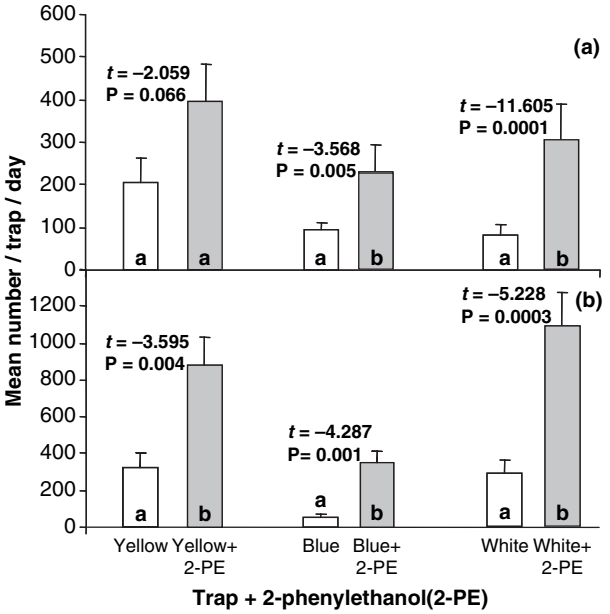

Fig. 5 Backtransformed mean number of *Astylus atromaculatus* in different coloured water traps, unbaited or baited with 2-phenylethanol (2-PE) released at 3 mg/day (a = 2000 season; b = 2001 season). Different letters inside each pair of bars (trap colour) indicate significant differences between means at the indicated significance levels according to the Behrens–Fisher *t*-test. Bars represent standard error.

cues and that trap efficiency increased with the addition of 2-phenylethanol lure, irrespective of trap colour. The results on colour preferences with unbaited water traps confirm previous laboratory studies (Esterhuizen 1997) in which *A. atromaculatus* was shown to prefer yellow visual cues to red and green. This suggests that *A. atromaculatus*, which is a flower forager and pollen feeder has, like other flower-inhabiting insects, an innate attraction to yellow (Wäckers 1994). Similar results were reported for another pollen beetle, *Meligethes* spp. in Europe (Láska et al. 1986; Blight and Smart 1999). However, attraction to yellow is not confided only to flower-feeding insects. Prokopy and Owens (1983) showed that a large number of herbivorous insects show preference for yellow. Astylus beetles also showed some attraction to white, which has also been reported for *Meligethes* spp. (Goos et al. 1976; Koštál 1992). Our results are also consistent with earlier work (Esterhuizen 1997), showing that *A. atromaculatus* was moderately attracted to blue discs.

Results also show that olfactory cues play a significant role in host attraction by *A. atromaculatus*. In general, the three antennally active components 2-phenylethanol, benzyl alcohol and linalool tested singly and combined with the yellow water traps increased trap captures, but this pattern was more pronounced in the tests carried out in 1999 than 2000. Twice as many total beetles were caught in the traps of 1999 as were trapped in 2000, reflecting the different seasonal levels of infestation by the beetle at the experimental site. Field trapping with yellow, blue and white water traps baited with 2-phenylethanol unambiguously demonstrated that this compound could be a promising attractant for further development for *A. atromaculatus* monitoring. Interestingly, 2-phenylethanol has been reported as an attractant for a number of insects including the onion and seed-corn flies *Delia* (*Hylemya*) *antiqua* and *Delia platura*, respectively (Ishikawa et al. 1983), cabbage looper moth *Trichoplusia ni*, (Haynes et al. 1991), green lacewing *Chrysoperla carnea* (Zhu et al. 1999) and pineapple beetle *Carpophilus humeralis* (Zilkowski et al. 1999).

2-Phenylethanol had the greatest effect on the relatively unattractive blue trap, confirming the importance of olfactory cues mediating *A. atromaculatus* attraction. This is similar to observations made on *Meligethes* spp. by Blight and Smart (1999). The latter authors showed that the visual × odour interaction indicated the magnitude of the odour effect to be dependent on the nature of the visual cue, and that lures had the greatest effect on traps of unattractive colour. Recently, Jönsson et al. (2007) reported that attraction of *M. aeneus* was influenced by the physiological state of the beetle and that over-wintered beetles were unresponsive to flower odours in the absence of the yellow colour.

In conclusion, this study has shown that 2-phenylethanol combined with a yellow trap could serve as a potential lure for monitoring populations of the pollen beetle. Further work will focus on testing blends of the antennally active components to obtain maximal attraction, as has been observed with the pollen beetle *M. aeneus*.

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